Final Report

Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method)

(Study code : GT13-00015)

January 2014

the way to trust KCL Korea Conformity Laboratories

BioConvergence Technology Laboratory

Statement

Study code : GT13-00015

Title : Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method)

This final report was written in Korean and translated into English.

This study has been performed in compliance with the principles of Good Laboratory Practices and test guidelines in following documents.

1. Standards of Good Laboratory Practice, National Institute of Environment Research (NIER)[Notice No. 2012-23 (revised 22th, Aug., 2012)]
2. Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER)[Notice No. 2012-23 (revised 22th, Aug., 2012)]
3. OECD Guideline for the Testing of Chemicals No. 423 'Acute Toxic Class Method' (Adopted 17th Dec., 2001)

The stated object in study protocol was achieved and there were no significant deviations from the aforementioned regulations that affected the quality or integrity of the study. Therefore the justification of all data in this study was confirmed. The information of the test substance was written from the document that the sponsor provided.

Kyu-Sup Ahn — Jan., 02, 2014

Kyu-Sup Ahn
Study Director
BioConvergence Technology Laboratory

Date

Jin Kyu Lee — Jan. 02, 2014

Jin-Kyu Lee
Managing Director
BioConvergence Technology Laboratory

Date

QUALITY ASSURANCE STATEMENT

Study No. : GT13-00015

Title : Acute Oral Toxicity Study of MWCNT in Spraque-Dawley Rats (Acute Toxic Class Method)

This study was subject to audit by the independent Quality Assurance Unit of KCL as indicated below. The findings of each audit were reported to the study director and management as prescribed by Standard Operating Procedures.

The final report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the final report accurately reflect the raw data produced during the study.

Audit phases and dates reported to the responsible personnel were as indicated below and these were based upon the audit records.

Phase Inspected	Date	Reports to Study Director	Reports to Management
Study Plan	2013. 02. 18	2013. 02. 18	2013. 02. 18
Storage of Test substance and vehicle	2013. 02. 26	2013. 02. 26	2013. 02. 26
Animal receipt	2013. 02. 28	2013. 02. 28	2013. 02. 28
	2013. 03. 12	2013. 03. 12	2013. 03. 12
Preparation of test substance	2013. 03. 05	2013. 03. 05	2013. 03. 05
Animal care and Administration	2013. 03. 05	2013. 03. 05	2013. 03. 05
Clinical sign	2013. 03. 19	2013. 03. 19	2013. 03. 19
Necropsy	2013. 03. 19	2013. 03. 19	2013. 03. 19
Raw data	2013. 04. 09	2013. 04. 09	2013. 04. 09
Final Report	2013. 04. 09	2013. 04. 09	2013. 04. 09

QA director : Song, Kyung Seuk Ph.D. Date 2013. 04. 09
Auditor, Quality Assurance

* signed original

Study Personnel

Formulation	Jae-Hyuck Sung*	**Date**	09 April 2013
Animal care	Min-Won Baek*	**Date**	09 April 2013
Necropsy & Pathology	Hye-Jin Kim*	**Date**	09 April 2013
Archiving	Hyo-Dong Kim*	**Date**	09 April 2013

* Signed original

Title	Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method)
Objective of Study	This study is performed to assess the acute oral toxicity and lethal dose 50 (LD_{50}) of MWCNT when the test substance is administered in single dose to rats.
Sponsor	Name : Bioconvergence Technology Laboratory Korea Conformity Laboratories Address : 7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea Tel. : 032-858-0011 Fax : 032-858-0020
Testing facility	Name : Bioconvergence Technology Laboratory Korea Conformity Laboratories Address : 7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea Tel. : 032-858-0011 Fax : 032-858-0020
Study Schedule	Animal acquisition (1st) : 28 February 2013 Animal acquisition (2nd) : 12 March 2013 Administration (1st) : 05 March 2013 Administration (2nd) : 12 March 2013 Necropsy (1st) : 19 March 2013 Necropsy (2nd) : 26 March 2013 Submission of final report : 02 January 2014
Archiving of study data	1) Archiving period : least 5 years after the study termination 2) Data : Study protocol, test substance data, animal acquisition data, raw data, final report and GLP documents 3) Storage room (1) Archive : CD, relevant document

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1. SUMMARY

This study has been performed to evaluate the lethal dose 50 (LD_{50}) and toxicity of the test substance MWCNT when it was administered in single oral dose to Sprague-Dawley (SD) female rats. The test substance was administrated by gavage at the dose level of 300 ㎎/㎏ in two steps (the first and second step). During the study period, dead animals, clinical signs, body weight changes and gross findings at necropsy were examined.

In all steps, no mortalities and unusual clinical signs were observed during the study period in all animals.
There were normal body weight gains in all animals.
At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

The third and forth step (2,000 ㎎/㎏) couldn't be conducted due to the solubility of the test substance (dispersion up to 1% in DPPC solution). Therefore, under these conditions, the acute oral lethal dose 50 (LD_{50}) of the test substance MWCNT is considered greater than 300 ㎎/㎏ body weight in female Sprague-Dawley rats.

2. TEST SUBSTANCE AND VEHICLE

1) Test substance (Annex 1)

(1) Name : MWCNT (Kumho : K-Nanos-100P)

(2) CAS No. : -

(3) Lot No. : -

(4) Received date : 25 January 2013

(5) Received quantity : 666.89 g (including container weight)

(6) Molecular weight : -

(7) Appearance : powder

(8) Purity : >90%

(9) Solubility : -

(10) Stability : -

(11) Storage condition : -

(12) Handling

① Wear protection equipments including gloves, mask, glasses and clothes.

② Keep the test substance in seal container.

③ Keep the test substance in low humanity and good ventilated condition.

(13) Supplier : Kumho Petrochmical Co., Ltd.

2) Vehicles

(1) Vehicle 1

① Name : 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

② Lot No. : 078K5203

③ CAS No. : 63-89-8

④ Molecular weight : 734.04

⑤ Received date : 21 March 2012

⑥ Received quantity : 1 g

⑦ Appearance : White powder

⑧ Purity : ≥99%

⑨ Storage condition : In freezer

⑩ Manufacturer : Sigma-Aldrich, Inc.

(2) Vehicle 2

① Name : Dulbecco's phosphate buffered saline (DPBS)

② Lot No. : 031M8307

③ CAS No. : -

④ Received date : 08 May 2012

⑤ Received quantity : 480 g
⑥ Appearance : White solid
⑦ Storage condition : Refrigeration
⑧ Manufacturer : Sigma-Aldrich, Inc.

(3) Vehicle 3
① Name : D-(+)-Glucose
② Lot No. : 071M0145V
③ CAS No. : 50-99-7
④ Received date : 28 August 2012
⑤ Received quantity : 1 ㎏
⑥ Appearance : White powder
⑦ Storage condition : At room temperature
⑧ Manufacturer : Sigma-Aldrich, Inc.

(4) Vehicle 4
① Name : Bovine serum albumin
② Lot No. : 750462
③ CAS No. : -
④ Received date : 06 April 2009
⑤ Received quantity : 100 g
⑥ Appearance : Yellow powder
⑦ Storage condition : Refrigeration
⑧ Manufacturer : Gibco

3) Justification for vehicle choice

The test substance was not dispersed in ordinary vehicles. So the DPPC solution (5.5 mM D-(+)-glucose+0.6 mg/ml Bovine serum albumin+0.01 ㎎/㎏ DPPC in DPBS) was selected as vehicle base on the reference. (Jin Sik Kim et al, 2011, Evaluation of biocompatible dispersants for carbon nanotube toxicity tests, Arch Toxicol, 204:723) At the result of solubility test, the test substance was dispersed equally up to 1% concentration in DPPC solution.

4) Storage and Treatment

The test substance was kept in a storage room (108-2). There is no mention about the store condition of the test solution because it was prepared in the morning of the administration day.

5) Formulation of the test solution

For the first and second steps (300 ㎎/㎏), 0.10 g of the test substance was dispersed evenly in DPPC solution to make a total volume of 10 ㎖. When it comes to the time of administration, the test solution was formulated and it was prepared three times. The homogeneity and stability tests of test solution were not performed because it was prepared and administrated in the morning of the administration day.

3. MATERIALS AND METHODS

1) Test animals

(1) Species and strains : Specific Pathogen Free(SPF) Sprague-Dawley(SD) rats

Used female rats were healthy young adults and they were nulliparous and non-pregnant.

(2) Producer and Supplier

ORIENT BIO INC. (Address; 143-1, Sangdaewondong, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea)

(3) Reason for selection of the species

SD rats have been applied widely in general toxicity tests as a suitable experimental animal for toxicity testing. In addition, sufficient raw data has been accumulated and is available for interpretation and evaluation of study results.

(4) Date of acquisition

1st acquisition : 28 February 2013

2nd acquisition : 12 March 2013

(5) Number of animals received : 7 female rats for each acquisition

(6) Age of animals received : 7 weeks

(7) Body weights on arrival

1st acquisition : 171.08~190.80 g

2nd acquisition : 188.39~209.37 g

(8) Quarantine and acclimation

Animals were acclimated for 5-12 days. Only animals with the best appearance were selected for the test after observation during the acclimation period, Animals were accepted based on the certification provided by the supplier (Annex 2).

(9) Age at the initiation of the administration : 8-9 weeks

(10) Body weights at the administration

G1(1st step 300 ㎎/㎏) : 180.06～198.15 g

G2(2nd step 300 ㎎/㎏) : 196.73～200.77 g

(11) Number of animals administered

Three female rats were used in each step. The test substance was administered to total six female rats.

(12) Grouping

Animals were weighed one day before the test substance administration and grouped to ensure a distribution of graded body weight.

(13) Identification of animals

Individual animals were identified by tail marking with an oily-ink felt-pen. Individual cages were distinguished by the individual card labeling. The record sheets provided at the entrance of the SPF animal room contained the study number, the study title, the duration of the SPF room use, the name of the study director and the names of study personnel.

(14) Disposal of remaining animals

They were treated by SOP of this testing facility

(15) Compliance with the guidelines of animal ethics

This study was approved by the IACUC of Korea conformity laboratory (approval number : IA13-00081).

2) Environmental and Housing Condition(Annex 3)

(1) Animal care room : Room 1 in the SPF animal facility area.

(2) Temperature and humidity : 22.2±0.6 ℃ and 45.8±3.7%RH

(3) Ventilation frequency : 10-15 air changes/hours

(4) Lighting cycle : 12 hours duration (lighting on at 8 a.m. and off at 8 p.m.)

(5) Lighting intensity : 282 Lux.

(6) Noise : 51.2 dB

(7) Concentration of ammonia : less than 5 ppm

(8) Housing

All animals were housed in wire mesh cages (250W × 350L × 180H ㎜) during quarantine, acclimation, administration and observation period. During the experiment, not more than 3 animals were housed in a cage and test animal cages were changed at grouping.

(9) Feeds and water

① Feeds

Radiation sterilized, solid laboratory animal feeds (Teklad Certified Irradiated

Global 18 % Protein Rodent Diet, Harlan Co. Ltd., USA) were provided *ad libitum.* DooYeol Biotech Co., Ltd. supplied feeds.

② Water

Incheon, Korea municipal tap water purified by reverse osmosis filtering system was provided *ad libitum* using water bottles.

③ Certification

The feed certification which was provided from the supplier and the water certification from national certificated inspection organization were referred to examine contamination (Annex 4, 5).

3) Method

(1) Administration

① Route of administration and reason for the selection

Application was oral by gavage to evaluate oral toxicity.

② Method of administration

Rats were fasted one night before the administration day to empty their stomach. An intubation cannula was used for the oral administration. All fasted test animals were fed approximately at three to four hours after test substance administration.

③ Frequency and duration of administration

Three times on the administration day, 2-3 hours interval, 10 ㎖/kg/once.

④ Calculation of dosing volume

Individual dosage was adjusted based on fasted body weight measured right before the administration. Dosing volume is 30 ㎖/kg body weight.

(2) Determination of dose level

Dose levels were determined in accordance with 'Guideline for the Testing of Chemical Hazards', National Institute of Environment Research (NIER)[Notice No. 2012-23 (revised 22th, Aug., 2012)] and the OECD Guideline for the Testing of Chemicals No. 423 'Acute Toxic Class Method' (Adopted 17th Dec., 2001). The dose level of first step was set as 300 ㎎/kg due to absence of toxicity information of the test substance.

(3) Group Description

Group	Sex	Number of animals	Identification of animals	Dose volume (㎖/kg)	Dose level (㎎/kg)
G1 (1st Step)	Female	3	G1-1~G1-3	30	300
G2 (2nd Step)	Female	3	G2-4~G2-6	30	300

(4) Study procedure

Each experimental group and step were designed in accordance with the study procedure diagram attached (Annex. 6). In the first step at the dose level 300 ㎎/㎏, there were no moribund or dead animals. So the dose level of the second step was set as 300 ㎎/㎏ and any moribund animals or mortalities were not observed. According to a study procedure diagram, the third and forth step must be performed as a 2,000 ㎎/㎏ dose level but this test was finished at second step because the volume of an abovementioned dose level was too much that the test solution couldn't be administrated. (volume : 200 ㎖/㎏, ∵ solubility : dispersion up to 1% in DPPC solution) The dose level of each step was decided on the result of observations for 6 days after the test substance administration.

(5) Observations and Examinations

① Clinical signs and mortalities

General clinical signs or mortalities of all treated animals were observed continuously during the first half-hour and the one hour from the administration time. After that, those animals were observed once hourly up to the first six hours on the administration day. From the next day, each animal was observed once every day up to 14 days after the administration.

② Body weight measurement

All individual animals were weighed before the administration and on 1, 7 and 14 days after the administration.

③ Necropsy and gross findings examination

On 14 day after the administration, all survival animals were anesthetized with CO_2 gas and terminated by exsanguination from the abdominal aorta and caudal vena cava. Complete post-mortem examinations were performed on all vital organs.

(6) Data analysis

Body weight changes of all animals in each step were analysed through tables and figures that were applied to the mean value and the standard deviations. Additional statistical assessment was not performed in data analysis. The LD_{50} value was classified according to the study procedure diagram of 'Guideline for the Testing of Chemical Hazards', National Institute of Environment Research (NIER)[Notice No. 2012-23 (revised 22th, Aug., 2012)] and the OECD Guideline for the Testing of Chemicals No. 423 'Acute Toxic Class Method'(Adopted 17th Dec, 2001).

4. RESULTS

1) The first step; 300 ㎎/㎏ (Table 1-3, Figure 1, Appendix 1-3)

No mortalities and unusual clinical signs were observed during the observation period in all animals.

There were normal body weight gains in all animals.

At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

2) The second step; 300 ㎎/㎏(Table 1-3, Figure 2, Appendix 1-3)

No mortalities and unusual clinical signs were observed during the observation period in all animals.

There were normal body weight gains in all animals.

At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

5. DISCUSSION AND CONCLUSION

This study has been performed to evaluate the lethal dose 50 (LD_{50}) and toxicity of the test substance MWCNT when it was administered in single oral dose to Sprague-Dawley (SD) female rats. The test substance was administrated by gavage at the dose level of 300 ㎎/㎏ in two steps (the first and second step). During the study period, dead animals, clinical signs, body weight changes and gross findings at necropsy were examined.

In the first and second step (300 ㎎/㎏), no mortalities and unusual clinical signs were observed during the study period in all animals.
There were normal body weight gains in all animals.
At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

The third and forth step (2,000 ㎎/㎏) couldn't be conducted due to the solubility of the test substance (dispersion up to 1% in DPPC solution). Therefore, under these conditions, the acute oral lethal dose 50 (LD_{50}) of the test substance MWCNT is considered greater than 300 ㎎/㎏ body weight in female Sprague-Dawley rats.

6. REFERENCES

1) Standards of Good Laboratory Practice, National Institute of Environment Research (NIER)[Notice No. 2012-23 (revised 22th, Aug., 2012)]

2) Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER)[Notice No. 2012-23 (revised 22th, Aug., 2012)]

3) OECD Guideline for the Testing of Chemicals No. 423 'Acute Toxic Class Method' (Adopted 17th Dec., 2001)

7. TABLES

Table 1. Mortalities and clinical signs of female rats

SUMMARY OF MORTALITIES AND CLINICAL SIGNS			
STUDY : GT13-00015			SEX : FEMALE
		GROUP(㎎/㎏)	
		G1(300)	G2(300)
MORTALITIES	N	0/3	0/3
	%	0	0
CLINICAL SIGNS	Normal	3/3	3/3

Number of dead animals / Number of animals examined

Table 2. Body weight changes of female rats

SUMMARY OF BODY WEIGHT CHANGES(g)								
STUDY : GT13-00015							SEX : FEMALE	
Day	GROUP(㎎/㎏)							
	G1(300)				G2(300)			
0	189.74	±	9.11	(3)	199.19	±	2.16	(3)
1	210.01	±	10.49	(3)	215.35	±	5.46	(3)
7	223.50	±	9.69	(3)	228.39	±	7.54	(3)
14	238.96	±	9.65	(3)	237.61	±	12.95	(3)

Mean±S.D (Number of animals)

Table 3. Gross findings of female rats

SUMMARY OF GROSS FINDINGS			
STUDY : GT13-00015			SEX : FEMALE
ORGAN	SIGN	GROUP(㎎/㎏)	
		G1(300)	G2(300)
All organs	Normal	3/3	3/3

Number of animals with the signs / Number of animals examined

8. FIGURES

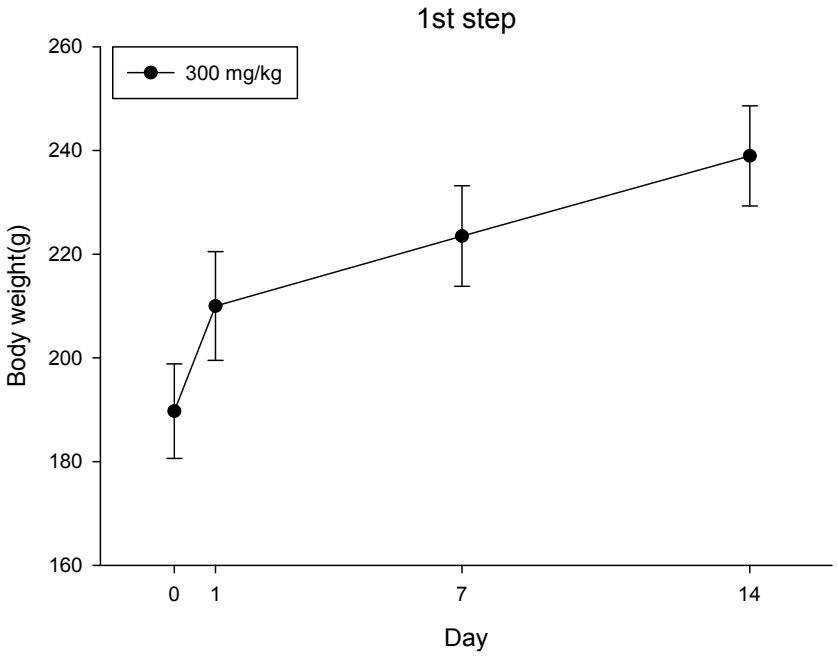


Figure 1. Body weight changes of female rat (1st step; 300 ㎎/㎏)

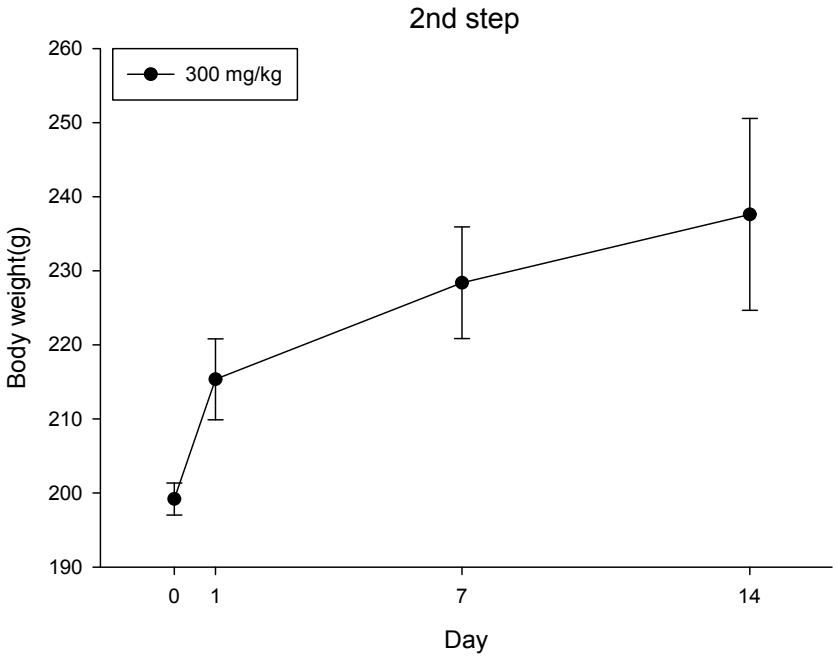


Figure 2. Body weight changes of female rat (2nd step; 300 ㎎/㎏)

9. APPENDICES

Appendix 1. Mortalities and clinical signs of female rats

INDIVIDUAL DATA OF MORTALITIES AND CLINICAL SIGNS

STUDY : GT13-00015 SEX : FEMALE

GROUP (mg/kg)	ANIMAL ID	DATE DOSED	OBSERVATIONS	TIME OCCURRED
G1 (300)	G1-1	05-Mar-2013	Normal	Day 0 - 14
			Terminal sacrifice	Day 14
	G1-2	05-Mar-2013	Normal	Day 0 - 14
			Terminal sacrifice	Day 14
	G1-3	05-Mar-2013	Normal	Day 0 - 14
			Terminal sacrifice	Day 14
G2 (300)	G2-4	12-Mar-2013	Normal	Day 0 - 14
			Terminal sacrifice	Day 14
	G2-5	12-Mar-2013	Normal	Day 0 - 14
			Terminal sacrifice	Day 14
	G2-6	12-Mar-2013	Normal	Day 0 - 14
			Terminal sacrifice	Day 14

Appendix 2. Body weight changes of female rats

INDIVIDUAL DATA OF BODY WEIGHT CHANGES(g)

STUDY : GT13-00015 SEX : FEMALE

GROUP (mg/kg)	ANIMAL ID	Day 0	Day 1	Day 7	Day 14	Gain[a]
G1 (300)	G1-1	180.06	197.96	213.12	230.12	50.06
	G1-2	191.01	217.12	225.05	237.52	46.51
	G1-3	198.15	214.94	232.32	249.25	51.10
	Mean	189.74	210.01	223.50	238.96	49.22
	S.D.	9.11	10.49	9.69	9.65	2.41
G2 (300)	G2-4	200.07	210.20	220.01	222.83	22.76
	G2-5	200.77	221.07	230.52	246.96	46.19
	G2-6	196.73	214.78	234.63	243.04	46.31
	Mean	199.19	215.35	228.39	237.61	38.42
	S.D.	2.16	5.46	7.54	12.95	13.56

a : Body weight gains between day 0 and day 14

Appendix 3. Gross findings of female rats

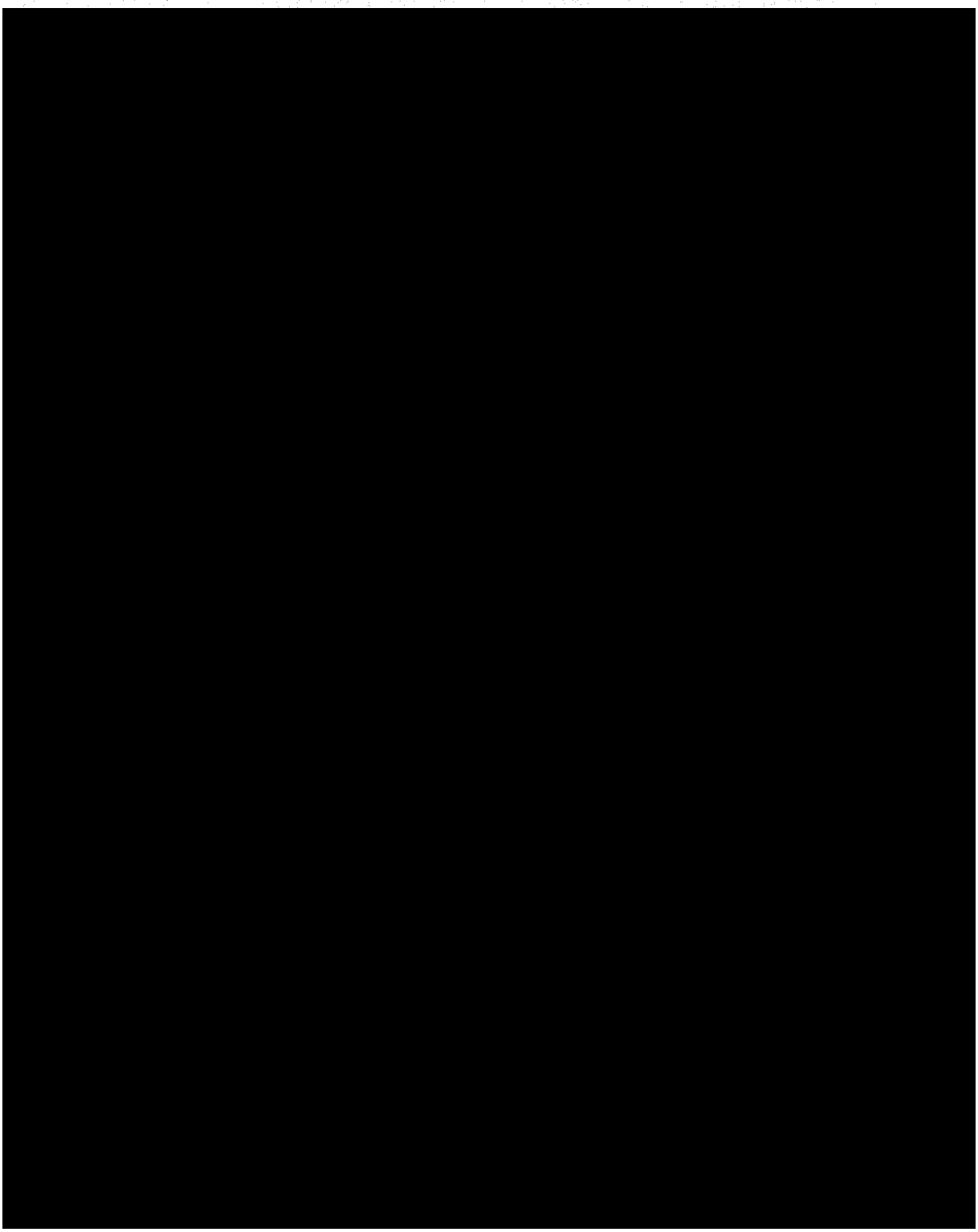
INDIVIDUAL DATA OF GROSS FINDINGS

STUDY : GT13-00015 SEX : FEMALE

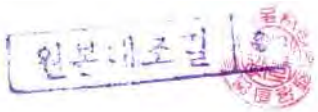
GROUP (mg/kg)	ANIMAL ID	FATE(DAY)	ORGAN	OBSERVATIONS
G1 (300)	G1-1	Terminal sacrifice(14)		No organ with gross findings
	G1-2	Terminal sacrifice(14)		No organ with gross findings
	G1-3	Terminal sacrifice(14)		No organ with gross findings
G2 (300)	G2-4	Terminal sacrifice(14)		No organ with gross findings
	G2-5	Terminal sacrifice(14)		No organ with gross findings
	G2-6	Terminal sacrifice(14)		No organ with gross findings

10.ANNEXES

Annex 1. Test substance chemical data sheet



Annex 2. Animal certification



CR Rodent Production

Rat VAF Report

Location: Orient Bio Inc. KP800 VAF Rat — **Sponsor: Orient Bio Inc.**

Colony: Crl:CD(SD) Colony # 28804 — **Reported: Sunday, October 28, 2012 at 20:54**

Summary Item	Primary Assay	Most Recent		Past 18 Months
		Year-Week	Positive / Tested	Positive / Tested
Virology				
SEND ae	MFIA	2012-41	0 / 8	0 / 48
PVM ae	MFIA	2012-41	0 / 8	0 / 48
SDAV ad	MFIA	2012-41	0 / 8	0 / 48
KRV ad	MFIA	2012-41	0 / 8	0 / 48
H1 ad	MFIA	2012-41	0 / 8	0 / 48
RPV ad	MFIA	2012-41	0 / 8	0 / 48
RMV ad	MFIA	2012-41	0 / 8	0 / 48
REO ae	MFIA	2012-41	0 / 8	0 / 48
RTV ad	MFIA	2012-41	0 / 8	0 / 48
LCMV ae	MFIA	2012-41	0 / 8	0 / 48
HANT ae	MFIA	2012-41	0 / 8	0 / 48
MAV ae	MFIA	2012-41	0 / 8	0 / 48
Microbiology				
B. bronchiseptica be	Culture	2012-41	0 / 8	0 / 48
CAR Bacillus ae	MFIA/PCR	2012-41	0 / 8	0 / 48
C. kutscheri ae	Culture	2012-41	0 / 8	0 / 48
H. bilis be	PCR	2012-41	0 / 8	0 / 48
H. hepaticus ae	PCR	2012-41	0 / 8	0 / 48
Helicobacter sp. be	PCR	2012-41	0 / 8	0 / 48
K. oxytoca ce	Culture	2012-41	0 / 8	0 / 48
K. pneumoniae ce	Culture	2012-41	0 / 8	0 / 48
M. pulmonis ae	MFIA	2012-41	0 / 8	0 / 48
P. multocida ce	Culture	2012-41	0 / 8	0 / 48
P. pneumotropica ce	Culture	2012-41	0 / 8	0 / 48
P. aeruginosa ce	Culture	2012-41	0 / 8	0 / 48
Salmonella spp. ae	Culture	2012-41	0 / 8	0 / 48
S. moniliformis af	PCR	2012-41	0 / 8	0 / 24
Strep. pneumoniae be	Culture	2012-41	0 / 8	0 / 48
Pneumocystis ("RRV") bd	MFIA	2012-41	0 / 8	0 / 48
Tyzzer's Disease ag	Exam	2012-41	0 / 8	0 / 48
Pathology				
Gross Exam ei	Exam, Histopathology	2012-41	0 / 8	0 / 48
Parasitology				
Ectoparasites ae	Exam	2012-41	0 / 8	0 / 48
Helminths ae	Exam	2012-41	0 / 8	0 / 48
Giardia sp. be	Exam	2012-41	0 / 4	0 / 24
Spironucleus sp. be	Exam	2012-41	0 / 4	0 / 24
Other Protozoa ce	Exam	2012-41	0 / 4	0 / 24
E. cuniculi ae	MFIA	2012-41	0 / 8	0 / 48

COLONY POLICY FOR POSITIVE RESULT: a = immediate termination, b = planned future recycle of the colony, c = no action.
TESTING SCHEDULE: d = screened every four weeks, e = screened quarterly, f = screened annually, g = screened quarterly by clinical exam.
i = results do not include incidental or strain related findings; significant findings would result in immediate termination of the colony.

Charles River RADS-ILIMS Report **Page 1 of 1**

Annex 2. Animal certification (continued)

CR Rodent Production

Rat VAF Report

Location: Orient Bio Inc. KP800 VAF Rat **Sponsor: Orient Bio Inc.**

Colony: Crl:CD(SD) Colony # 28804 **Reported: Monday, March 4, 2013 at 1:22**

Summary Item	Primary Assay	Most Recent		Past 18 Months
		Year-Week	Positive / Tested	Positive / Tested
Virology				
SEND ae	MFIA	2013-05	0 / 8	0 / 48
PVM ae	MFIA	2013-05	0 / 8	0 / 48
SDAV ad	MFIA	2013-05	0 / 8	0 / 48
KRV ad	MFIA	2013-05	0 / 8	0 / 48
H1 ad	MFIA	2013-05	0 / 8	0 / 48
RPV ad	MFIA	2013-05	0 / 8	0 / 48
RMV ad	MFIA	2013-05	0 / 8	0 / 48
REO ae	MFIA	2013-05	0 / 8	0 / 48
RTV ad	MFIA	2013-05	0 / 8	0 / 48
LCMV ae	MFIA	2013-05	0 / 8	0 / 48
HANT ae	MFIA	2013-05	0 / 8	0 / 48
MAV ae	MFIA	2013-05	0 / 8	0 / 48
Microbiology				
B. bronchiseptica be	Culture	2013-05	0 / 8	0 / 48
CAR Bacillus ae	MFIA/PCR	2013-05	0 / 8	0 / 48
C. kutscheri ae	Culture	2013-05	0 / 8	0 / 48
H. bilis be	PCR	2013-05	0 / 8	0 / 48
H. hepaticus ae	PCR	2013-05	0 / 8	0 / 48
Helicobacter sp. be	PCR	2013-05	0 / 8	0 / 48
K. oxytoca ce	Culture	2013-05	0 / 8	0 / 48
K. pneumoniae ce	Culture	2013-05	0 / 8	0 / 48
M. pulmonis ae	MFIA	2013-05	0 / 8	0 / 48
P. multocida ce	Culture	2013-05	0 / 8	0 / 48
P. pneumotropica ce	Culture	2013-05	0 / 8	0 / 48
P. aeruginosa ce	Culture	2013-05	0 / 8	0 / 48
Salmonella spp. ae	Culture	2013-05	0 / 8	0 / 48
S. moniliformis af	PCR	2013-05	0 / 8	0 / 32
Strep. pneumoniae be	Culture	2013-05	0 / 8	0 / 48
Pneumocystis ("RRV") bd	MFIA	2013-05	0 / 8	0 / 48
Tyzzer's Disease ag	Exam	2013-05	0 / 8	0 / 48
Pathology				
Gross Exam ei	Exam, Histopathology	2013-05	0 / 8	0 / 48
Parasitology				
Ectoparasites ae	Exam	2013-05	0 / 8	0 / 48
Helminths ae	Exam	2013-05	0 / 8	0 / 48
Giardia sp. be	Exam	2013-05	0 / 8	0 / 28
Spironucleus sp. be	Exam	2013-05	0 / 8	0 / 28
Other Protozoa ce	Exam	2013-05	0 / 8	0 / 28
E. cuniculi ae	MFIA	2013-05	0 / 8	0 / 48

COLONY POLICY FOR POSITIVE RESULT: a = immediate termination; b = planned future recycle of the colony; c = no action.
TESTING SCHEDULE: d = screened every four weeks; e = screened quarterly; f = screened annually; g = screened quarterly by clinical exam.
1 = results do not include incidental or strain related findings; significant findings would result in immediate termination of the colony.

Charles River RADS-ILIMS Report **Page 1 of 1**

Annex 3. Environmental certification of animal care room

Certification of Environment for animal breeding room

Study No.	GT13-00015
Title	Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats(Acute Toxic Class Method)
SPF Room No.	SPF #1 Animal Room
Period of animal Breeding	2013 -02 - 28 ~ 2013 -03 - 26

Breeding environment condition

Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	22.2±0.6 ℃	
Humidity	50±20 %RH	45.8±3.7 %RH	
Luminous intensity	150~300 Lux	282 Lux	
Noise	60 dB less than	51.2 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck

2013-12-31

Annex 4. Laboratory animal diet certification

Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-073012MA
Date of Manufacture 07/30/12
Report Date 08/15/12

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2012.08.16 11:01:11 -05'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.00
Fat	6.15
Fiber	3.06
Moisture	11.70
Ash	5.46
Calcium	1.03
Phosphorus	0.73

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.13	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.46	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Annex 5. Certification of water analysis

TEST REPORT

1. No : PC12-00576
2. Client
   - ○ Name : Korea Conformity Laboratories(Incheon)
   - ○ Address : #7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea
   - ○ Date of Receipt : Sep. 25, 2012
   - ○ Date of Issued : Oct. 30, 2012
3. Use of Report : Submission
4. Test Sample : Drinking Water (Animal room)
5. Test Results

Reissuance (R1)
Date : 2012.10.30

----- Refer to the next page -----

Affirmation	Tested by Name : Hyoung jun Seok Seok.	Technical Manager Name : Sang Cheul Lee S.C. Lee

Our report apply only to the standards or procedures identified and to the sample(s) tested unless otherwise specified.
The test results are not indicative of representative of the qualities of the lot from which the sample was taken or of apparently identical or similar products.

Korea Conformity Laboratories President Jae Bin Song Jae Bin Song

Address : 153-803 459-28, Gasan-Dong, Geumcheon-Gu, Seoul, Korea 82-2-2102-2500
Result Inquiry : 82-2-2102-2598

QP-20-01-07(1)

Annex 5. Certification of water analysis (continued)

No : PC12-00576

TEST REPORT

Test Items	Units	Limitations	LOQ	Test Results
Total colony counts	CFU/mL	Less than 100	0	34
Total coliforms	-/(100mL)	Not detected	-	Not detected
E-Coli	-/(100mL)	Not detected	-	Not detected
Lead	mg/L	Less than 0.01	0.005	Not detected
Arsenic	mg/L	Less than 0.01	0.005	Not detected
Selenium	mg/L	Less than 0.01	0.005	Not detected
Cadmium	mg/L	Less than 0.005	0.002	Not detected
Bolon	mg/L	Less than 1.0	0.01	Not detected
Copper	mg/L	Less than 1.0	0.008	Not detected
Zinc	mg/L	Less than 3.0	0.002	0.003
Iron	mg/L	Less than 0.3	0.05	Not detected
Manganese	mg/L	Less than 0.3	0.005	Not detected
Aluminium	mg/L	Less than 0.2	0.02	Not detected
Mercury	mg/L	Less than 0.001	0.001	Not detected
Fluoride	mg/L	Less than 1.5	0.15	Not detected
Nitrate nitrogen	mg/L	Less than 10	0.1	0.2
Chloride	mg/L	Less than 250	0.4	Not detected
Sulfate	mg/L	Less than 200	2	Not detected
Diazinon	mg/L	Less than 0.02	0.000 5	Not detected
Parathion	mg/L	Less than 0.06	0.000 5	Not detected
Fenitrotion	mg/L	Less than 0.04	0.000 5	Not detected
Dichloromethane	mg/L	Less than 0.02	0.002	Not detected
1,1,1-Trichloroethane	mg/L	Less than 0.1	0.001	Not detected
Benzene	mg/L	Less than 0.01	0.001	Not detected
Toluene	mg/L	Less than 0.7	0.001	Not detected
Ethylbenzene	mg/L	Less than 0.3	0.001	Not detected
Xylene	mg/L	Less than 0.5	0.001	Not detected
1,1-Dichloroethylene	mg/L	Less than 0.03	0.001	Not detected
Tetrachlorocarbon	mg/L	Less than 0.002	0.001	Not detected
Tetrachloroethylene	mg/L	Less than 0.01	0.001	Not detected
Trichloroethylene	mg/L	Less than 0.03	0.001	Not detected
1,2-Dibromo-3-Chloropropane	mg/L	Less than 0.003	0.001	Not detected

- page 2 of 3 -

QP-20-01-08(1)

Annex 5. Certification of water analysis (continued)

No : PC12-00576

TEST REPORT

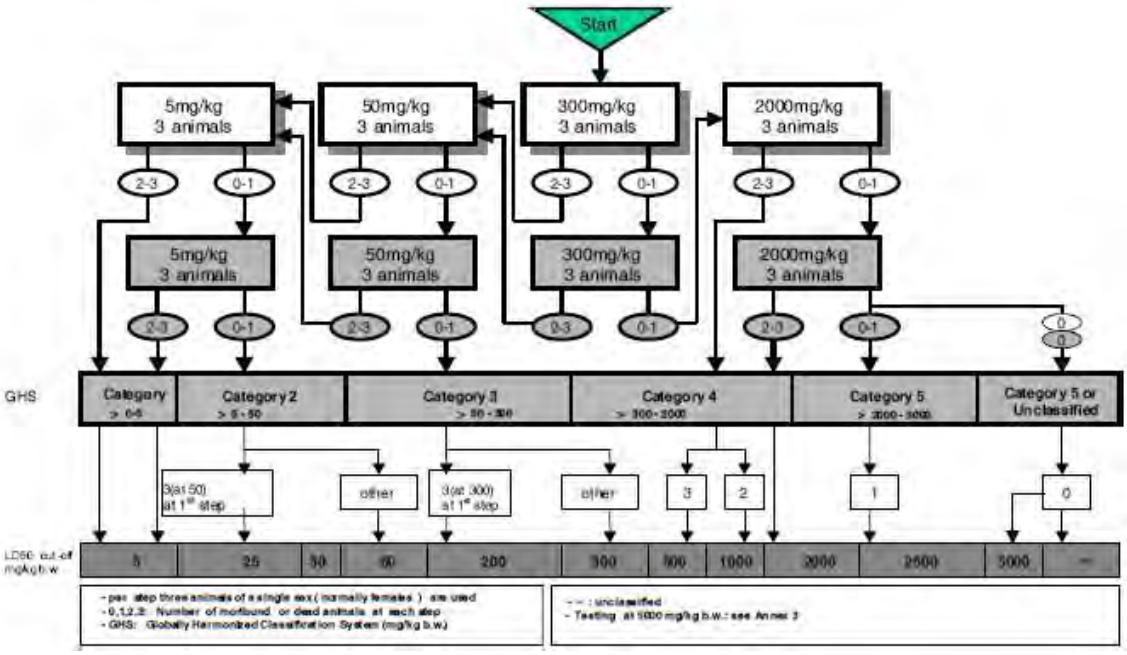
Test Items	Units	Limitations	LOQ	Test Results
Carbaryl	mg/L	Less than 0.07	0.005	Not detected
Chromium	mg/L	Less than 0.05	0.003	Not detected
Ammonia nitrogen	mg/L	Less than 0.5	0.01	Not detected
Phenol	mg/L	Less than 0.005	0.005	Not detected
Detergent	mg/L	Less than 0.5	0.1	Not detected
Cyanide	mg/L	Less than 0.01	0.01	Not detected
pH	-	5.8 ~ 8.5	-	6.4
Turbidity	NTU	Less than 1	0.02	0.16
Color	degree	Less than 5	1	Not detected
Taste	-	Free	-	Pass
Odor	-	Free	-	Pass
Hardness	mg/L	Less than 300	1	Not detected
Consumption of $KMnO_4$	mg/L	Less than 10	0.3	0.9
Total solids	mg/L	Less than 500	2	4
Test method	Notification No.2012-143 of the Ministry of Environment			

—— End of Report ——

- page 3 of 3 -

QP-20-01-08(1)

Annex 6. Study procedure diagram



Annex 7. Study protocol amendment sheet-Translated

Study protocol (amendment · deviation) sheet

<This study protocol amendment sheet was written in Korean and translated into English.>

Study Code	GT13-00015	Study period	2013. 02. 26 - 2013. 04. 26
Title	Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method)		

Amendment · Deviation Contents	Amendment · Deviation Reason
The date of 1st animal acquisition was changed **Before amendment** (4) Date of acquisition 1st acquisition : 26 February 2013 **After amendment** (4) Date of acquisition 1st acquisition : 28 February 2013	**1. Reason** ◦ There was a dead animal as well as cannibalism in a animal box on arrival. Therefore, all animals in that box were euthanized. ◦ Besides, all animal in other boxes arrived at the same time were also euthanized. **2. The effect of amendment on this study** ◦ There are no affects on the study because all test animals were reacquired. ◦ Despite changed date of acquisition, the acclimation period of animals was corresponded with the study protocol. cf) Study protocol 7) Quarantine and acclimation Animals are acclimated for more than 5 days. Changed acclimation period : 28 February 2013 - 4 March 2013 (5 days)

Study director	: Kyu-Sup Ahn	Management director	: Jin-Gyu Lee
Date	: 26 February 2013	Date	: 26 February 2013
QAU	: Kyung-seuk Song	Date	: 26 February 2013
Sponsor	: Not application		

Annex 8. KCL GLP certificate

지정번호 (Certification No.) 제2008-4호		화학물질 유해성 시험기관 지정서 GLP Certificate
①	시험기관	한국생활환경시험연구원 안전성평가본부
	Test Facility Name	Korea Environment and Merchandise Testing Institute Bio-Safety Evaluation Headquarters
②	소 재 지	인천광역시 연수구 송도동 7-44
	Address	7-44, Songdo-Dong, Yeonsu-Gu, Incheon, 406-840, Korea
③	대 표 자	김 창 로
	President	Chang-Ro Kim
④	운영책임자	유 일 재
	Test Facility Management	Il-Je Yu
⑤	시험의 범위	- 급성경구독성시험, 유전독성시험(복귀돌연변이시험, 염색체이상시험, 소핵시험). (유효기간 : 2006년 3월 31일부터). 끝. - 급성피부자극성 및 부식성시험, 급성안자극성 및 부식성시험, 급성흡입독성시험. (유효기간 : 2007년 4월 17일부터). 끝. - 아급성독성시험, 피부감작성시험. (유효기간 : 2008년 8월 25일부터). 끝.
	Test Scope	- Acute oral toxicity, Genetic Toxicity(Ames test, Chromosome abberation test, Micronucleus test) (Validation : since Mar. 31, 2006). - Acute dermal irritation/corrosion, Acute eye irritation/ corrosion, Acute inhalation toxicity (Validation : since Apr. 17, 2007). - Subchronic toxicity, Skin sensitization (Validation : since Aug. 25, 2008).

「유해화학물질관리법」 제14조, 같은 법 시행령 제12조 및 같은 법 시행규칙 제10조제2항에 따라 화학물질 유해성 시험기관(GLP시험기관)으로 지정합니다.

It is hereby certified that the test facility was inspected by the national compliance monitoring authority regarding compliance with the Principles of Good Laboratory Practice.

Issue date 2008년(year) 8월(month) 25일(date)

국립환경과학원장 인

President, National Institute of Environmental Research

Annex 8. KCL GLP certificate (continued)

(뒤 쪽)-1

<변경사항>

일자	내용	확인
2009. 5. 20	운영책임자 변경 : 유 일 재 (Il-Je Yu) 에서 송 경 석 (Kyung-Seuk Song) 으로 변경	
2009. 11. 16 (국문)	시험의 범위 : 급성경피독성시험, 어류급성독성시험 (유효기간 : 2009년 11월 16일 부터) 끝.	
〃 (영문)	Test Scope : Acute dermal toxicity, Fish: acute toxicity (Validation : since Nov. 16, 2009).	
2010. 8. 2	대표자 변경 : 김 창 로 (Chang-ro Kim) 에서 오 태 식 (Taeshik Oh) 로 변경	G L P 확 인
2010. 8. 2	기관명 변경 "한국산업융합연구원 생명융합기술센터 바이오융합본부"로 변경 *영문명 (Bioconvergence Technology Division, Korea Conformity Laboratories)	G L P 확 인
2011. 9. 9	운영책임자 변경 : 송 경 석 (Kyung-Seuk Song) 에서 이 진 규 (Jin Kyu Lee) 으로 변경	G L P 확 인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인
2010. 12.	정기서류평가 결과, GLP기준을 준수하고 있음 (GLP Compliance)	G L P 확 인

Annex 8. KCL GLP certificate (continued)

화학물질유해성시험기관 지정서
제2008-4호

(뒤 쪽)-2

<변경사항>

일자	내용	확인
2011. 9. 9	기관명변경: "한국건설생활환경시험연구원 바이오융합단"으로 변경 (Bioconvergence Technology Department, Korea Conformity Laboratories)	G L P 확 인
2011. 11. 3	대표자 변경 : 오태식 (Taeshik Oh)에서 송 재빈 (Jae Bin Song)으로 변경	G L P 확 인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인

Annex 9. Quality assurance statement-Original

신 뢰 성 보 증 확 인 서

시험번호 : GT13-00015

시 험 명 : Sprague-Dawley 랫드를 이용한 MWCNT의 급성경구독성시험 (독성등급법)

이 보고서에 기술된 시험을 독립적으로 아래와 같이 시험과정 단계별로 점검하였으며 각 점검결과를 표준작업지침서에 따라 시험책임자와 운영책임자에게 통보 및 보고하였다.

본 시험은 국립환경과학원 고시 제2012-23호(2012년 08월 22일) '화학물질유해성시험연구기관의 지정 등에 관한 규정', 동 고시 별표5 화학물질유해성시험방법 제4장 제2항 "급성경구독성시험(독성등급법)" 및 OECD Guideline for the Testing of Chemicals No. 423 'Acute Toxic Class Method'(Adopted 17th Dec., 2001)에 따라 수행되었으며, 보고서 작성 방법 및 결과의 기술이 시험 실시 과정에서 발생한 시험기초자료를 바탕으로 정확히 반영되었음을 확인하였다.

점 검 내 용	실 시 일	시험책임자에게 통보일	운영책임자에게 보고일
시험계획서 점검	2013. 02. 18	2013. 02. 18	2013. 02. 18
시험물질 및 대조물질	2013. 02. 26	2013. 02. 26	2013. 02. 26
동물입수	2013. 02. 28 2013. 03. 12	2013. 02. 28 2013. 03. 12	2013. 02. 28 2013. 03. 12
시험물질조제	2013. 03. 05	2013. 03. 05	2013. 03. 05
동물사육 및 투여	2013. 03. 05	2013. 03. 05	2013. 03. 05
증상관찰 및 측정	2013. 03. 19	2013. 03. 19	2013. 03. 19
부검	2013. 03. 19	2013. 03. 19	2013. 03. 19
시험기초자료	2013. 04. 09	2013. 04. 09	2013. 04. 09
최종보고서 점검	2013. 04. 09	2013. 04. 09	2013. 04. 09

한국건설생활환경시험연구원 바이오융합연구소
신뢰성보증책임자 송경석 (인)

2013 년 04 월 09 일

Annex 10. Study personnel-Original

시험관계자 서명

시험물질 조제	성 재 혁 시험물질 조제분석 책임자	날짜	2013. 04. 09
동물관리	백 민 원 동물관리 책임자	날짜	2013. 04. 09
부검 및 병리	김 혜 진 병리 책임자	날짜	2013. 04. 09.
자료보관	김 효 동 자료보관 책임자	날짜	2013. 04. 09.

Annex 11. Study protocol amendment sheet-Original

시험계획서 (변경 · 이탈)기록지

시험번호	GT13-00015	시험기간	2013. 02. 26 ~ 2013. 04. 26
시험제목	Sprague-Dawley 랫드를 이용한 MWCNT의 급성경구독성시험(독성등급법)		

변 경 · 이 탈 사 항	변 경 · 이 탈 사 유
동물입수일 변경 **변경전** 동물 입수일(1차) : 2013 년 02 월 26 일 **변경후** 동물 입수일(1차) : 2013 년 02 월 28 일	1. 사유 ◦ 입수 시 사망동물이 존재하였고 해당 사육상자에서 카니발리즘(cannibalism) 현상이 관찰되어 모든 동물을 안락사 처리함. ◦ 또한 동일한 시기에 함께 입수된 다른 동물들에 대해서도 안락사 처리함. 2. 시험에 미치는 영향 ◦ 새로운 동물을 재입수하여 시험에 이용하므로 동물에 의해 시험에 미치는 영향은 없을 것으로 판단됨. ◦ 변경된 날짜로 입수하여도 시험계획서에 부합하므로 시험에 미치는 영향은 없을 것으로 판단됨. cf) 시험계획서 : 시험은 입수 후 5 일 이상의 순화를 거치며, 순화기간 중 일반증상을 관찰하여 건강한 동물만을 시험에 사용한다. 변경된 순화기간 : 2013 년 02 월 28 일 ~ 2013 년 03 월 04 일(5 일)
시험책임자 : 안 규 성 (인)	운영책임자 : 이 진 기 (인)
날 짜 : 2013년 02 월 26 일	날 짜 : 2013 년 02 월 26 일
신뢰성보증부서 : 품질보증부 (인) 날짜 : 2013 년 02 월 26 일	
시험의뢰자 : 해당사항없음 (인) 날짜 : 년 월 일	

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